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THE BACTERIAL INTEGRITY OF CELLOIDIN AND PARCHMENT MEMBRANES.*

DAVID DUKE TODD.
(From the Bacteriological Laboratory of the University of Chicago.)

INTRODUCTION.

THE celloidin membrane occupies an important place in the bacteriological technique of today because of its perfect osmotic properties, and its accepted imperviousness to the passage of bacteria. The osmotic properties are well known. The bacterial integrity for all micro-organisms has been assumed from the results of isolated experiments with certain bacteria, which were found not to pass through the membrane. It is the purpose of this paper to give the results of experiments made with the object of testing the bacterial integrity of the celloidin, and also of the parchment membrane, to various types of bacteria, especially to the intestinal group.

Historical.—The celloidin sac was introduced for bacteriological purposes by Morpurgo and Tirelli,[1] who exploited it as a ready means for the cultivation of the tubercle bacillus. Their method was to place a piece of infected tissue into a celloidin sac, which was then sealed and placed either under the skin or preferably into the peritoneal cavity of a rabbit. They found that the sac soon filled with cell-free serum, and in several weeks there appeared an abundant growth of the tubercle bacillus. Sacs were allowed to remain in the peritoneal cavity of rabbits upward of two months without any of the rabbits showing signs of infection. This demonstrated the impermeability of the celloidin membrane for the tubercle bacillus.

Metchnikoff, Roux, and Salimbeni[2] further advanced the use of the celloidin sac in their study of the toxin production of the cholera spirillum. They filled celloidin sacs with inoculated broth, and introduced them into the peritoneal cavity of guinea-pigs. The animals succumbed on the third to the fifth day, with all the signs of cholera poisoning. At autopsy, the sacs were found intact and no cholera spirilla could be demonstrated in the peritoneal fluid, heart's blood, or viscera.

Two years later Nocard and Roux[3] ingeniously used the celloidin sac in the demonstration of the cause of pleuro-pneumonia of cattle. The results of these investigators, in the successful cultivation of an almost ultra-microscopic organism, by the use of the celloidin sac, seemed to be ample as evidence confirming the conclusions of Morpurgo and Tirelli, and Metchnikoff, Roux, and Salimbeni that the celloidin membrane offers

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[1] *Archives ital. de biologie*, 1892, 18, p. 187; *Centralbl. f. Bakt., Ref.*, 1893, 13, p. 74.

[2] *Ann. de l'Inst. Pasteur*, 1896, 10, p. 257.

[3] *Ibid.*, 1898, 12, p. 240.

an impermeable barrier to bacteria as well as to body cells, while it permits of perfect osmosis.

Nocard also used the sac in his experiments on the transformation of the human into the avian type of tubercle bacillus, by placing the bacilli in celloidin sacs inserted into the peritoneal cavity of a fowl. He found that the tubercle bacilli did not pass through the sac wall.

Up to this time the celloidin sac had been used for intraperitoneal placement only. Carnot and Fournier[1] were the first to use it in tube experiments, made in their study of the pneumococcus and its toxins. They found that the pneumococcus occasionally past through the sac as evidenced by turbidity in the outside fluid.

McCrae[2] used the sacs in a study of the agglutinins obtained with paratyphosus and enteritidis cultures. To control his technic of making the sacs he incubated them, after inoculation, for 24 hours in broth. Finding no turbidity in the outside fluid, he held that the sacs were intact, and ready for intraperitoneal placement. That the bacteria did not escape from the sacs, when in the peritoneal cavity of the rabbit, he held to be shown by the observation that upon the insertion of the inoculated sacs agglutinins appeared and gradually increased. On the removal of the sacs, they began to disappear.

Jordan, Russell, and Zeit[3] and Russell and Fuller[4] used the celloidin sac in an exhaustive study of the longevity of the typhoid bacillus. No definite experiments were conducted by these investigators with the object of testing the integrity of celloidin for the typhoid bacillus, since owing to the legal exigencies under which the experiments were made the results of previous experiments had to be accepted at their face value.

It thus appears that the basis for the belief of the bacterial integrity of the celloidin membrane rests upon experiments made, chiefly with the tubercle bacillus and the cholera spirillum. On the other hand the occasional turbidity found in the outside broth in the experiments of Carnot and Fournier, working with the pneumococcus, suggests either the presence of imperfections in the sacs, or that the pneumococcus could actually grow through the celloidin membrane.

Technic.—Novy[5] first described a method of making the celloidin sac. Previous investigators, Morpurgo and Tirelli and Metchnikoff, Roux, and Salimbeni, failed to describe their technic. The method of Novy consisted in repeatedly dipping a glass tube into a solution of celloidin and allowing it to dry when sufficiently coated. It was then carefully folded back on itself from the upper extremity and completely inverted. This gave a well-formed sac which was firmly attached by ligatures to a piece of glass tubing. After sterilization in broth the sac was inoculated, and closed by sealing the glass tube in the flame. Ruffer and Crendiropoulo,[6] and Eyre[7] changed the technic somewhat relative to the method of separating the celloidin from the glass tube. They dipped the coated tube alternately for a few seconds into strong alcohol and then into water. The water and alcohol diffusing through the celloidin gradually loosened it from the glass and the sac was readily stript off. A glass tube was then inserted into the neck of the sac and fastened and closed by a method similar to that of Novy. Eyre strengthened the junction between the sac and glass tube by a ligature

[1] *Archiv. de méd. expér.*, 1900, 12, p. 357.
[2] *Jour. Exper. Med.*, 1901, 5, p. 635.
[3] *Jour. Infect. Dis.*, 1904, 1, p. 650.
[4] *Ibid.*, 1906. Supplement No. 2, p. 40.
[5] *Bacteriological Laboratory Handbook*, 1898.
[6] *Brit. Med. Jour.*, 1900, 2, p. 1305.
[7] *Bacteriological Technique*, 1902, p. 279.

before he sealed it. The sac was fixt in an empty tube, and the whole sterilized by dry heat at 150° C. Gorsline[1] molded sacs over a piece of tubing which had a small opening left in the end. The celloidin was then stript off by forcing water through the tube. Prudden[2] earlier used a similar method in which he forced air through the tube instead of water. The sac was attached to a glass tube by heating, and closed by sealing the glass in the flame.

McCrae reported the successful use of the gelatin capsule over which to mold the celloidin sac. His method was later modified by Harris, who used a piece of tubing with a constriction near the end which he fixt to the cap of the capsule by slightly heating the tubing, and bringing it into contact with the cap. The entire capsule was then dipt into celloidin and when sufficiently coated, was allowed to dry. The gelatin was melted by hot water and pipetted off, the sac being closed by sealing the glass tube in the flame.

In these various methods a piece of glass tubing remains as part of the sac and, when placed in the peritoneal cavity of the experimental animal, it acts more or less as an irritant. Harvey[3] has obviated this trouble by a further modification of the gelatin-capsule method of molding the celloidin sac. He made an opening into the body of the capsule large enough to permit the passage of a piece of glass tubing which was heated and attached to the inside of the cap, He then dipt the capsule into a paraffin bath allowing an even coat of paraffin to harden on the outside of the capsule, which was then dipt into celloidin until of sufficient thickness. When the last layer had set, the whole was dipt into chloroform, which dissolved the paraffin and left the gelatin capsule free from the celloidin. The sac was then dipt into alcohol and then into water so that the gelatin was softened and could be readily withdrawn, leaving the celloidin sac. This he closed by filling the neck with aseptic wool and covering it over with melted paraffin. The neck was then wiped dry with a piece of absorbent cotton soaked in alcohol, and sealed with celloidin.

The most serviceable technic for making any desired size of celloidin sac is the method described by Kellerman[4] and supplemented by Frost.[5] The method consists in pouring the celloidin solution into a glass tube of the desired size and, after the coating has air hardened, in shrinking it from the glass by pouring in water. This method is easy of application, rapid, and can be utilized to make a sac of any desired size.

In my work the method of Kellerman was followed. The celloidin used is an eight per cent solution by weight of equal parts of absolute alcohol and sulfuric ether.[6] A large tube served for molding, which gives a sac with a capacity of about 5 c.c. The celloidin is poured into the tube till about one-fourth full and, while rotating the tube, the celloidin is poured back into the stock bottle leaving an even coating free from bubbles. The tube is rotated for a few minutes in a horizontal position and then the celloidin is hardened by a current of air forced into the tube from a foot-bellows. After the celloidin is dry enough so that it does not stick to the finger, and before it has started

[1] *Science*, 1902, 15, p. 375.

[2] Cited by Harris, *Johns Hopkins Hosp. Bull.*, 1902, 13, p. 112.

[3] *Centralbl. f. Bakt.*, 1908, 46, p. 285.

[4] *Jour. Applied Micros.*, 1902, 5, p. 2038.

[5] *Amer. Pub. Health Assoc. Rep.*, 1902, 28, p. 536.

[6] It is important that these proportions be followed for if an excess of alcohol is used the sacs dry slowly and unevenly, while if too much ether is used the sacs dry too rapidly and are hard to handle because of cracking.

to shrink from the side of the tube, a second coating of celloidin is added as above. When dry, the sac is filled with water and removed by pulling on it and separating the celloidin from the wall of the tube by a dull instrument. It is fitted to a glass tube of slightly smaller bore, ligated with heavy thread, and sealed with celloidin. After this it is filled with water which prevents further drying. It was found that if the sacs were allowed to dry in the air until they were greatly creased and of a whitish color they readily broke and were unfit for use.

The celloidin sacs used in animal work were molded in the ordinary test-tube cut off to a length of two inches. Upon removal of the sac, a glass tube of 3 mm. bore was inserted, and the top of the sac drawn down tight with a wrapping of heavy thread, the ends being left long. This so fashioned the celloidin that when the glass tube was pulled out it left a sac with a small opening and a wide flange at the neck. This flange is readily flattened out and the edges cut off short. The sac is filled with water and dropped into a test-tube and in this condition can be kept in stock. For use the sac is filled, immersed in broth contained in a large test-tube, and sterilized. The sterile sac is withdrawn from the tube by means of the long thread and so held by an assistant.[1] A small amount of the broth is removed by means of a sterile pipette, and the broth in the sac inoculated by a loop of the desired culture. Care should be taken not to touch the sides of the sac during the process. After inoculation the sac is closed by pulling on the threads and tying. To seal, the sac is seized at the neck with a pair of sterile forceps, the water removed by absorbent cotton soaked in absolute alcohol, and several coatings of celloidin applied. The sac is repeatedly washed with sterile water and returned to the tube of broth ready for intraperitoneal adjustment.

GROWTH OF BACTERIA THROUGH THE CELLOIDIN MEMBRANE.

Experiments, as we have seen, have been made which are the foundation of the assumption of the integrity of the celloidin membrane for all bacteria. These experiments were with *B. tuberculosis* and *Sp. cholerae*. Other bacteria were not used, and it has been my purpose to find the status of the integrity of the celloidin membrane for other bacteria, principally those of the intestinal group.

Each sac, used in the following experiments, was tested for leaks by immersing it in water and blowing into the sac as hard as possible. If no leaks were detected by this air test, the sac was filled with a saturated egg-albumin solution (freshly prepared) and immersed up to the glass neck in as small an amount of water as possible contained in a 500 c.c. graduate, the sac being held in place by cotton packed around the glass tube. At the end of 48 hours the outside water was tested for the presence of albumin by the buiret and Millon tests. If these were negative the integrity of the sac was held established. The egg albumin was then carefully washed out and the sac was ready for use.

[1] A pair of needle forceps clamped to a ring stand is serviceable.

Treating the celloidin membrane with chloroform and alcohol seemed to make it harder to the touch. It is generally held that this treatment makes the membrane more durable. Accordingly, a series of sacs was hardened, after being air dried, with chloroform for five minutes followed by alcohol for five minutes. As to the durability the treated sacs, although they felt harder and seemed to be a little tougher than the untreated, they behaved in similar experiments exactly as did the latter.

The permeability of the treated and untreated sacs was tested with sodium chloride. Sacs were filled with distilled water containing enough added salt to make the chlorin test 140 parts per million. The content of the sacs was tested for chlorin at intervals as expressed in Table 1.

TABLE 1.

CHLORIN FOUND IN CELLOIDIN SACS AFTER PERIODS IN SALT SOLUTION (140 PARTS PER MILLION).

	UNTREATED		TREATED	
	Series A	Series B	Series A	Series B
45 minutes	14.4	19.2	9.6	4.8
3 hours, 15 minutes	43.2	52.8	19.2	14.4
18 hours	43.2	67.2	43.2	48.0
24 "	52.8	115.2	91.2	91.2
31 "	100.8	124.8	96.0	96.0
43 "	120.0	115.2	115.2	115.2
52 "	120.0	120.0	124.8	135.4
72 "	124.8	135.4	129.6	135.4

From these results it appears that the variation in the time required to establish an equilibrium in the untreated and treated sacs is so slight that, as far as the permeability for the chlorides is concerned, the hardening of the sacs has little if any effect. One significant fact is brought out in the length of time required to establish an equilibrium. This speaks for the thickness of the celloidin sacs used which, according to the prevailing ideas, should offer an effectual barrier to the passage of bacteria.

To determine the bacterial integrity of the celloidin membrane, sacs were filled with broth and immersed in broth contained in ordinary commercial pepton bottles. Each sac was held in place by cotton well packed about the neck, and the tube of the sac also plugged with cotton. The outfit, container and sac, was then sterilized by heating in the autoclav for 10 minutes. Upon cooling, a

part of the broth in the sac was removed with aseptic precautions so that the broth in the sac was about 1 cm. below the junction of the celloidin and glass. Then the sac was raised so that the celloidin-glass junction was above the surface of the broth in the container. This procedure obviated the exposure to the growth of the bacteria of possible thin portions about the junction of the celloidin and glass. Each outfit, after sterilization and the necessary after-manipulations, was placed in the incubator for at least 24 hours and, if no turbidity was observed in the fluid either within or without the sac, it was ready for inoculation. The sac was inoculated with a loop of a 24-hour culture of the chosen micro-organism, and outfit was placed in the thermostat. Observations were made at frequent intervals for any turbidity in the outside broth. If turbidity was observed a small amount of the broth was withdrawn and plated in appropriate dilutions. Cultures were isolated from the colonies and the bacterium identified. In all cases where the outside broth became turbid the species of bacteria with which the sac had been inoculated, was isolated. The results are expressed in Table 2.

TABLE 2.

GROWTH OF BACTERIA THROUGH CELLOIDIN MEMBRANE.

	Average Period of Incubation	Recovery from Outside Broth	Number of Experiments	Earliest Time of Recovery*
B. prodigiosus	24 hours	+	10	.....
B. pyocyaneus	3 days	+	2	.....
B. coli	24 hours	+	7	9 hours
B. aerogenes	24 hours	+	4	9 "
B. chol. suis	3 days	+	2	.....
B. enteritidis	3 "	+	2	.....
B paratyphosus	3 "	+	2	.....
B. typhosus	24 hours	+	8	4 hours
B. dysenteriae	48 "	+	2	.....
B. alkaligenes	24 "	+	2	.....
B. vulgaris	36 "	+	2	.....
B. cloacae	24 "	+	2	.....
Staphylococcus	6 days	o†	6	.....
Streptococcus	6 "	o	5	.....
Pneumococcus	8 "	o§	4	.....
B. subtilis	11 "	o	3	.....
B. anthracis	11 "	o	3	.....
B. pseudodiphtheriticus	13 "	o	5	.....
Sp. cholerae	20 "	o‡	8	.....
Sp. metchnikovi	20 "	o	2	.....

* If a period before 18 hours.

† In one case a pure culture of staphylococcus was recovered from the outside broth on the fifth day. The air and albumin tests were negative.

‡ In one case a pure culture of the spirillum was recovered from outside broth. The air and albumin tests were negative.

§ Milk was used as a culture medium and not broth.

A study of these results demonstrates conclusively that certain species of bacteria, of which the intestinal group is a type, readily

grow through the celloidin membrane. On the other hand, the cocci—staphylococcus, streptococcus, and pneumococcus—the diphtheria group, the anthrax group, and the spirilla—of cholera and metchnikovi—do not usually grow through. There were two single exceptions in the case of the staphylococcus and the cholera spirillum. The results of the experiments with the members of the intestinal group and *B. prodigiosus* and *B. pyocyaneus*, show that the current view of the bacterial integrity of the celloidin membrane is not tenable for all bacteria. Some are able to grow through and others are not.

It would appear at first glance that the motility of the bacteria was a factor in the ability of certain micro-organisms to grow through the celloidin membrane. The cocci, non-motile, did not grow through, while *B. typhosus*, motile, readily grew through. However, *B. aerogenes*, non-motile, readily grew through and *Sp. cholerae*, very actively motile, did not grow through in 20 days. Thus the explanation does not lie in motility but rather appears to be a peculiar property of certain bacteria, motile or non-motile.

To show that the growth of the bacteria through the celloidin membrane was not because of leaks in the sacs, the following control was made. Each outfit, after the test of the growth of the bacteria in the sac had been completed, was sterilized in the autoclav, taking the precaution to have the sac well immersed in the broth. After sterilization the sac was removed from the container and tested for leaks by the air test. If this was negative the sac was filled with a saturated egg-albumin solution and immersed in water. If the air test was positive or if after four days the outside water showed the presence of albumin, the entire experiment was discarded. By this procedure there was an absolute check upon the results of the experiment as far as the detection of any defects present in the sacs which would permit of the ready passage of bacteria in any way than through the actual thickness of the celloidin membrane.

THE DIRECT PASSAGE OF BACTERIA THROUGH THE CELLOIDIN MEMBRANE.

Having found that certain bacteria were able to grow through the celloidin membrane, experiments were undertaken to learn, if

possible, whether this group of bacteria could pass directly through in the absence of a pabulum for growth.

This determination is of importance in view of the technic that was used by Jordan, Russell, and Zeit, and later by Russell and Fuller in the study of the longevity of the typhoid bacillus. Their method was to seed celloidin and parchment sacs with the typhoid bacillus and then expose the sacs in sewage or running water, examining the sacs at frequent intervals for the presence of typhoid bacilli. In accordance with the results of previous experimenters the bacterial integrity was assumed to hold in periods up to several weeks.

Johnson[1] questioned the bacterial integrity of the parchment membrane in the light of his work with parchment sacs at the Columbus Sewage Testing Station. He found that parchment sacs of initial and continued integrity, as far as dialyzing properties were concerned, permitted the escape of *B. coli* when immersed in distilled water. In flowing sewage large numbers of motile bacteria including *B. coli* passed through the sac wall in a comparatively short time. He did not experiment with the typhoid bacillus but says, "That if other less motile forms could pass through the walls of unpunctured parchment sacs under such conditions, there is no room for reasonable doubt, regarding the ability of the typhoid-bacillus to act in a similar manner." This observer did not experiment with celloidin.

The plan of my experiments to test the direct passage of the bacteria, was to use distilled water in container and sac, to sterilize the outfit in the autoclav, and to seed the content of the sac with a loop of the desired culture. This was very carefully removed from the agar-slant so as not to carry over any culture medium which would favor the growth of the bacteria. The outfit was then placed in a vessel of running tap-water by which a temperature was maintained ranging from 8°–12° C. This simulated, as far as temperature was concerned and its effect on the bacteria, the condition if the bacteria had been exposed directly in running water. Three species of bacteria were used—*B. typhosus*, *B. pyocyaneus*, and *B. prodigiosus*. Withdrawals of 5 c.c. of the outside water were made at intervals, run into broth, and incubated. If there was growth, plates were

[1] *Jour. New Eng. Water Works Assoc.*, 1905, 19, p. 508

made, colonies picked and identified,[1] with the results as expressed in Table 3.

TABLE 3.

DIRECT PASSAGE OF BACTERIA THROUGH THE CELLOIDIN MEMBRANE.

	AVERAGE SEEDING	TIME OF RECOVERY FROM OUTSIDE WATER		NUMBER OF EXPERIMENTS
		Earliest	Latest	
B. prodigiosus	540,000	16 hours	48 hours	10
B. pyocyaneus	.....	27 "	60 "	3
B. typhosus	286,000	14 "	67 "	8

From this it is shown that *B. prodigiosus*, *B. pyocyaneus*, and *B. typhosus* are able to pass directly through the celloidin membrane and that, too, without an increase or growth in the sacs. In the experiments with *B. typhosus* the number of colonies found on the plates when 1 to 2 c.c. of the outside water was directly plated ranged from 20 to 50.

Not alone in experiments with a pure culture of these bacteria but in the presence of the bacteria found in tap-water were *B. prodigiosus* and *B. typhosus* found to pass readily through the celloidin membrane. Sacs were filled and immersed in tap-water but not sterilized. In experiments with *B. prodigiosus*, plates were made with appropriate dilutions to see if the water contained any red-pigment producers. If none were found the sacs were seeded with *B. prodigiosus* and if red colonies appeared on plates of the outside water, it was taken to show the passage of *B. prodigiosus* through the sac wall. Controls showed that there were no red-pigment producers in the tap-water kept in containers during the same period of time. In the case of *B. typhosus* the sacs were seeded and the outside water plated at intervals on Conradi-Drigalski medium. Typical colonies were picked at periods ranging from 46 hours to three days, and identified by cultural tests and the agglutination reaction (1:1000). These are positive results which further go to prove the power of certain bacteria to pass directly through the celloidin membrane, in the presence of other micro-organisms.

In my first experiments the method was to seed the celloidin sac

[1] The identification of *B. prodigiosus* and *B. pyocyaneus* was made from the cultural reactions and pigment-production. The identification of *B. typhosus* was made from the different culture tests and the agglutination reaction (dilution 1:1000).

and test the outside fluid for the presence of the bacterium. Experiments were now made to test the passage of bacteria from without into the sac. Russell and Fuller report negative results of experiments in which sacs were immersed in running sewage and tests made for the presence of sewage bacteria in the sacs.

Celloidin sacs, filled with sterile water, were immersed in fresh sewage contained in a large vessel, and the content of the sacs tested at frequent intervals for the presence of bacteria. A comparative study made of sacs filled with sewage and the outside water (sterile tap-water) tested for bacteria, and sacs filled with sterile water and immersed in sewage (Table 4) shows that the bacteria of the intestinal flora readily pass through the membrane, either from within outward or from without inward, the time required in the former condition being apparently somewhat longer than in the latter.

TABLE 4.

PASSAGE OF SEWAGE BACTERIA THROUGH THE CELLOIDIN MEMBRANE.*

SEWAGE INSIDE OF SAC.

SERIES	TIME OF RECOVERY OF BACTERIA FROM OUTSIDE WATER		
	24 hours	48 hours	108 hours
I	+	....	....
II	o	+	....
III	+	....	..

SEWAGE OUTSIDE OF SAC

SERIES	TIME OF RECOVERY OF BACTERIA FROM INSIDE WATER		
	24 hours	48 hours	108 hours
I	+	....	....
II	o	o	+
III	o	+	....

* *B. alkaligenes* and *B. coli* were the predominant bacteria which passed through, as recovered from the plates.

A very striking result observed in the experiments was the ability of the typhoid bacillus to pass through the celloidin membrane. It was recovered from the outside broth as early as four hours after the sac was seeded. In the case of the colon bacillus the earliest period of recovery was nine hours. The difference in the penetrating power of the two bacteria was observed by Cambier[1] who found that the typhoid bacillus passed through the Chamberland bougie when

[1] *Comptes rend. de l'Acad. de sci.*, 1901, 132, p. 1442.

immersed in broth in a period of 18–20 hours, while *B. coli* and other forms took a longer period. This fact he made the basis of a method suggested for the isolation of the typhoid bacillus.

I carried out a small series of experiments using the idea of Cambier for the isolation of typhoid bacilli from stools, but instead of using the Chamberland bougie, used the celloidin sac.

Stools were obtained from patients having typical symptoms of typhoid fever and a positive agglutination reaction, and celloidin sacs, filled and immersed in sterile broth, were inoculated with a loop of the feces and incubated. Withdrawals of the outside fluid were made after the sixth hour after inoculation at intervals of two hours if possible. The object was to obtain the organism which first passed through the sac wall. The broth was plated in blood glucose agar, and a plan of identification followed, according to the system of Epstein.[1] From 27 samples of stools, of 10 different patients in the first to the third week of the disease, no typhoid bacilli were recovered. *B. alkaligenes* was found to be the predominant organism in about 70 per cent of the samples and an unidentified bacillus, which is very actively motile, gram-negative, and does not ferment the ordinary sugars in about 30 per cent of the samples (Table 5). *B. coli* was found to pass through in all cases. There is no doubt that typhoid bacilli were abundant in the stool but in the experiments probably did not pass through the celloidin membrane before other forms.[2]

TABLE 5.

ISOLATION OF THE TYPHOID BACILLUS FROM FECES.

CASE	NUMBER OF SAMPLES	PREDOMINANT BACTERIUM		*B. coli* FOUND
		B. alkaligenes	Unidentified Bacillus	
1	3	3	...	3
5	2	2	...	2
6	2	...	2	2
7	3	1	2	3
38	3	3	...	3
40	2	1	1	2
44	4	4	...	4
69	1	1	...	1
71	3	...	3	3
72	4	4	..	4

[1] *Amer. Jour. Med. Sci.*, 1908, 136, p. 190.

[2] Further work is to be carried on along somewhat different lines with the object of thoroughly testing the celloidin membrane as a basis of a method for the isolation of the typhoid bacillus from stools. I wish to take this opportunity to thank Professor E. R. Le Count for his many kindnesses shown and Drs. Rogers and Speidell of the Cook County Hospital for their services in obtaining the samples.

THE BACTERIAL INTEGRITY OF PARCHMENT SACS.

This type of diffusible membrane is also held to be impervious to the passage of bacteria. Sacs were made out of the best parchment tubing obtainable, and sealed according to the method of Jordan, Russell, and Zeit. Many leaks were found in the sacs, it being almost impossible in a series of eighteen to find one that was negative to the albumin test. On testing, *B. prodigiosus* was found to pass readily through the parchment wall.

The non-dependable integrity of the ordinary parchment tubing was recognized by Russell and Fuller, and they used the diffusion shells of Schleicher and Schüll. I tested six of these shells, 38×85 mm. They were fitted to a glass tube of approximate bore, ligated and the junction sealed with celloidin. Each shell, upon testing, was negative to the air and albumin tests. They were then filled and immersed in broth. After sterilization they were seeded with *B. typhosus* and in each the outside broth was turbid at the expiration of 24 hours of incubation. A pure culture of *B. typhosus* was recovered. Each shell was sterilized and tested for leaks by the air and albumin tests and found negative.

These results clearly demonstrated that *B. typhosus* grows readily through the special parchment diffusion shells. Further, in view of the previous results with celloidin sacs, there is no room for doubt that the typhoid bacillus can also pass directly through the parchment shells in the absence of material for growth.

THE BACTERIAL INTEGRITY OF CELLOIDIN SACS IN THE ANIMAL BODY.

The extensive use of the celloidin sac for intraperitoneal placement in animal work has been largely in experiments with the tubercle bacillus and the cholera spirillum. The experimental results left no question but that the celloidin sac was impervious to the passage of these bacteria. The tube experiments with *Sp. cholerae*, cited in Table 2, further demonstrate the bacterial integrity of the celloidin membrane for this micro-organism. However, the bacterial integrity of the celloidin sacs for members of the intestinal group of bacteria in animal experimentation does not seem probable in view of my previous results.

McCrae,[1] working with *B. enteritidis* and *B. paratyphosus* in a study of their agglutinins, maintained that the sacs completely imprisoned the bacteria from the observation that the agglutinins fell when the sacs were removed. The question now arises, not as to the passage of agglutinogenic substance through the sac, but the probability of a slow escape of bacteria.

Sacs for intraperitoneal adjustment in rabbits were made as previously outlined. Their capacity ranged from 1½ to 3 c.c. The presence of leaks, it is readily seen, could not be determined by the air and albumin tests as was done in the case of the large sacs. However, an equally good test method was used. Twenty-four sacs were made and filled with broth. They were then divided into two lots. Each sac of one series was inoculated with staphylococci, which, it will be remembered, were found not to pass through the celloidin membrane (Table 2). The sacs were then sealed and returned to the tubes of broth and incubated. Each sac of the other lot was inoculated with *B. typhosus*, sealed, and returned to tubes of broth. Seven were incubated, and the remaining five were aseptically introduced into the peritoneal cavity of five rabbits.

Incubation of the 12 sacs inoculated with staphylococci showed growth in the outside fluid in two tubes. In one a bacillus was found, but no coccus, showing a contamination; and in the other tube there was a pure culture of staphylococcus. The outside broth of the remaining 10 sacs remained sterile, which was held clearly to establish the success of the method used in making the sacs without defects. Of the seven sacs inoculated with *B. typhosus* and incubated, all showed turbidity in the outside broth in less than 24 hours, with recovery of pure culture in each case.

The results of the experiments on the rabbits were as follows: One rabbit died from accidental injury. Three were anesthetized and as much peritoneal fluid removed, under aseptic precautions, as possible.

RABBIT 1.—Two to three c.c. of peritoneal fluid was removed 17½ hours after placement of the celloidin sac. Examination of a hanging-drop showed one to three motile organisms to a fluid. The peritoneal fluid was run into broth, well shaken, and plated on Conradi-Drigalski medium. Typical colonies were picked and identified, by cultural tests and agglutination reactions, to be *B. typhosus*.

[1] *Jour. of Exper. Med.*, 1901, 5, p. 635.

RABBIT 4.—*B. typhosus* recovered from peritoneal fluid 70 hours after placement of sac.

RABBIT 5.—*B. typhosus* recovered from peritoneal fluid 23 hours after placement of sac.

RABBIT 2.—This animal was bled at intervals and the serum tested for agglutinins by the microscopic tests. On the third day agglutination took place in a dilution 1:100, the fifth day 1:1,000, the seventh day 1:5,000, the 10th day 1:1,000, the 23d day 1:500.

The recovery of the typhoid bacillus from the peritoneal fluid of the rabbit not only adds further to the demonstration of the power of the bacillus to penetrate the celloidin membrane, but calls attention to the importance of this factor in animal work with certain bacteria. The rapid rise of agglutinins, from the use of the inoculated celloidin sac, suggests a feasible method of immunizing an animal against *B. typhosus*.

CONCLUSIONS.

The celloidin membrane, contrary to the accepted view of today, is not impervious to the passage of all bacteria.

The group of intestinal bacteria, and also *B. prodigiosus* and *B. pyocyaneus*, readily pass through celloidin sacs either by growth or direct passage.

The cocci—pneumococcus, staphylococcus, streptococcus—diphtheria and anthrax groups, and the spirilla—cholerae and metchnikovi—do not pass through celloidin membrane.

The bacterial permeability of the parchment sac is undoubtedly the same as that of the celloidin sac.

The results of experiments relative to the longevity of the typhoid bacillus, as determined by the use of the celloidin or parchment sacs, require retesting because of the partial escape of the bacilli from the sac.

In animal experiments with *B. typhosus*, when introduced in celloidin sacs into the peritoneal cavity, the factor of passage of the bacilli through the celloidin membrane must be considered in interpreting results. This is very probably true for all members of the intestinal group.

I wish to take this opportunity of thanking Professor E. O. Jordan for direction and suggestion during the course of this work.